Modi-1 a novel cancer vaccine targeting citrullinated vimentin and enolase

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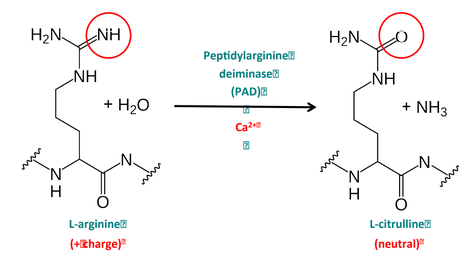
INTRODUCTION

- CD4 T cells are potent effectors but CD4 responses to self antigens are often attenuated.
- Cellular stress induces autophagy which leads to modification of proteins recognised by the immune system (3). One such modification is citrullination (cit).
- In the absence of inflammation, immunity is regulated, whereas in its presence CD4 responses to modified self-antigens are stimulated (2).
- Cancer cells citrullinate proteins (4). Citrullinated proteins in cancer cells include ubiquitous cytoskeletal protein Vimentin and glycolytic enzyme α-Enolase.
- Stressful conditions in tumour microenvironment and inflammation leads to presentation of modified peptides on MHC class II which is a target for CD4 T cells. We have shown that these can be harnessed for tumour therapy (5).
- In this study we assess the efficacy of a peptide vaccine targeting citrullinated vimentin and enolase (Modi-1) plus adjuvant (1) for tumour therapy in mouse models and its suitability for translation into the clinic.



Citrullination. A modification that occurs within stressed cells. Peptidylarginine deiminase (PADs) enzymes are activated and convert arginine to citrulline by altering the positively charge aldimine group (=NH) group of arginine to the neutrally charged ketone group (=O) of citrulline.

Modi-1 peptides stimulate Th1 responses in healthy human donors

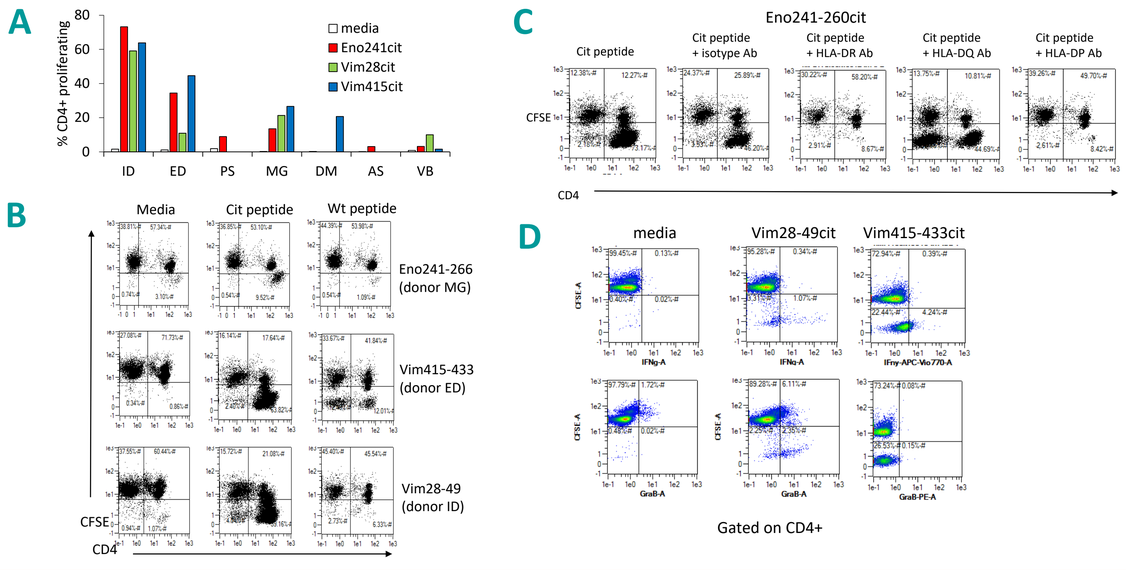


Figure 1.
Proliferation of PBMCs from healthy donors (depleted of CD25+ cells) assessed by CFSE dilution at day 11 after stimulation with 10μg/ml citrullinated or wild type peptides (A). Example data showing phenotyping of proliferating cells (B), blocking of proliferation by HLA-DP and HLA-DR antibodies (C) and IFNγ/granzyme B staining on proliferating cells (D).

Table 1. Donor HLA types

donor	A	B	C	DR	DQ	DP
MG	2	7,41	7,17	7,13,52a,53a	2,3	1,**4**
AS	3,24	15,27	2,3	4,53a	3	**4**,9
ID	1,2	8,44	5,7	3,15,51a,52a	2,6	1,**4**
VB	1,32	8,15	7	3,13	2,6	ND
ED						
DM	1	8	7	3	2	1,**4**
PS	2,29	44,51	ND	7,11	2,3	**4**,5

Modi-1 peptides mixed with TLR agonists stimulates IFNγ responses but IFA stimulates IL-10 in HLA-DR4 and HLA-DP4 transgenic mice

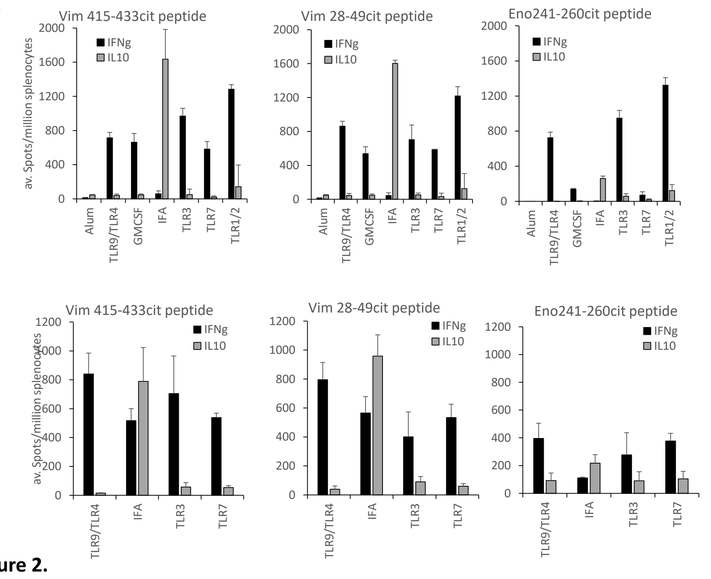


Figure 2.
Ex vivo IFNγ and IL-10 elispot responses from HLA-DR4 (A) or HLA-DP4 (B) transgenic mice immunised on days 0,7 & 14 with 10nmol citrullinated vimentin 28-49, 415-433 and Enolase 241-260 peptides mixed with TLR9/4 (CpG/MPLA), TLR3 (Poly I:C), TLR7 (Imiquimod), TLR1/2, GMCSF, Alum or IFA adjuvants. Responses analysed at day 20. Data normalised against background control.

Modi-1 peptides with TLR9/4 agonists promote tumour therapy

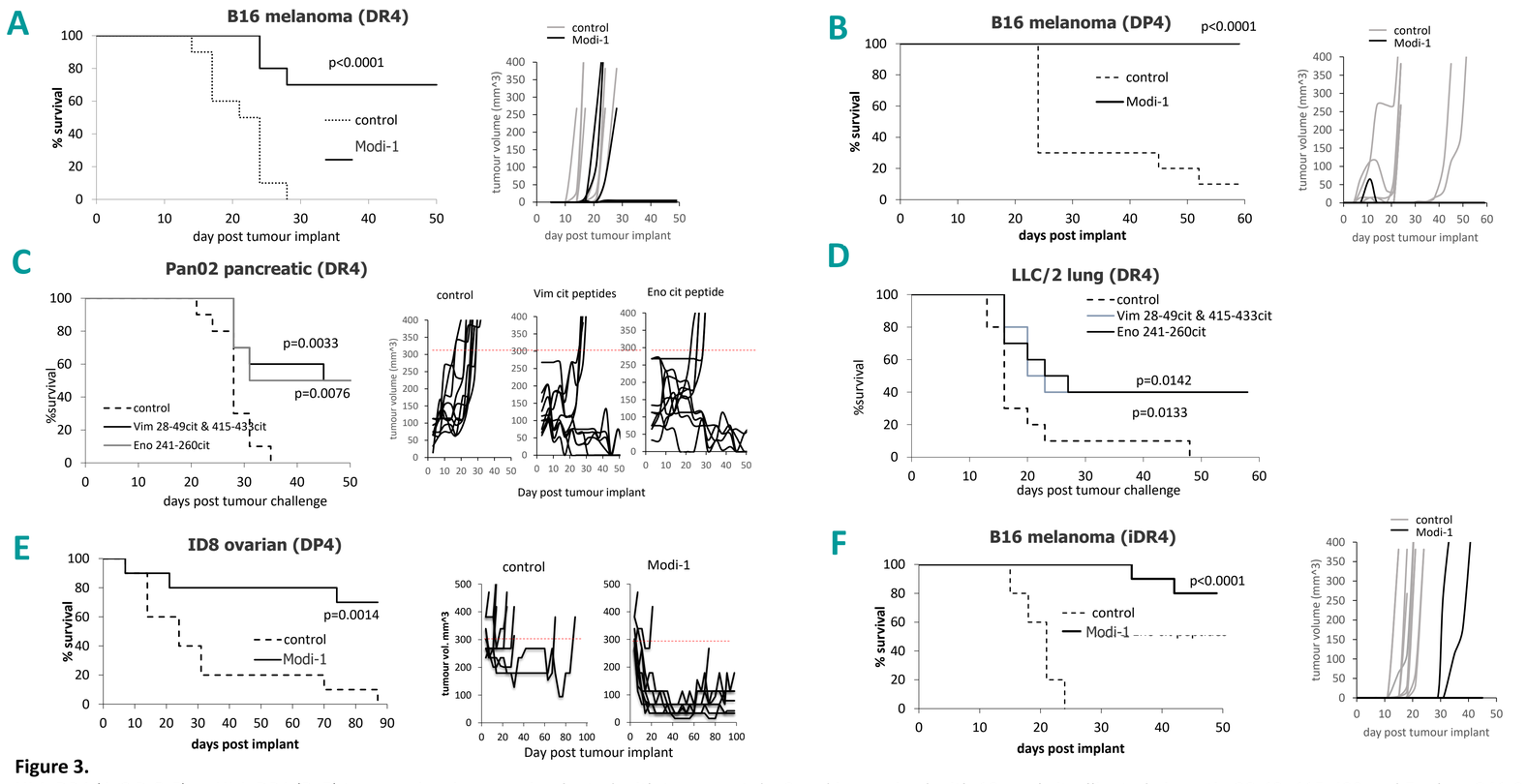


Figure 3.
HLA-DR4 (A,C,D & F) or HLA-DP4 (B, E) transgenic mice were implanted with tumour at day0 and immunised with 10nmol citrullinated vimentin 28-49, 415-433 and Enolase 241-260 peptides mixed with CpG/MPLA (TLR9/4) at day 4, 7 & 11. Survival and tumour growth was monitored. Tumours were engineered to constitutively express DR4 (DR4) or DP4 (DP4) or to express DR4 under IFNγ inducible promoter (iDR4).

Immunisation with IFA stimulates IL-10 responses detectable within 2 days that do not protect from tumour challenge

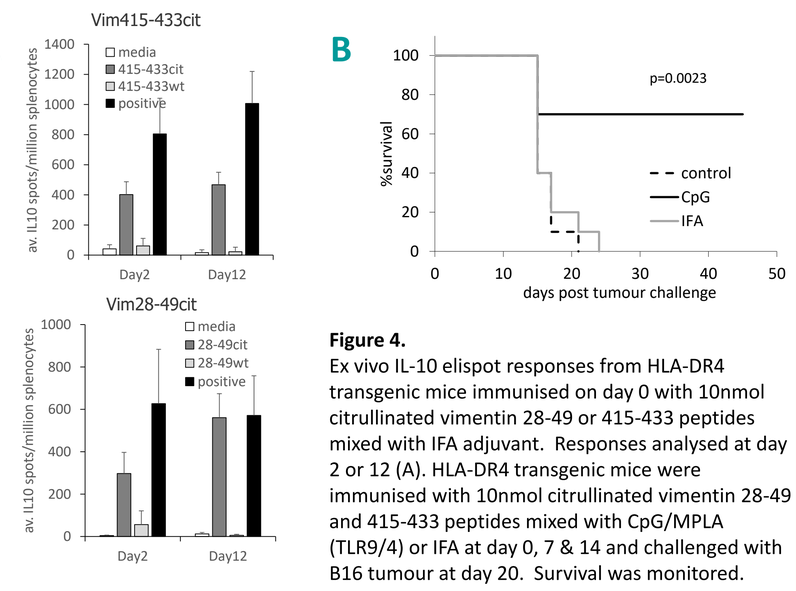


Figure 4.
Ex vivo IL-10 elispot responses from HLA-DR4 transgenic mice immunised on day 0 with 10nmol citrullinated vimentin 28-49 or 415-433 peptides mixed with IFA adjuvant. Responses analysed at day 2 or 12 (A). HLA-DR4 transgenic mice were immunised with 10nmol citrullinated vimentin 28-49 and 415-433 peptides mixed with CpG/MPLA (TLR9/4) or IFA at day 0, 7 & 14 and challenged with B16 tumour at day 20. Survival was monitored.

Ovarian and TNBC tumours express citrullinated vimentin

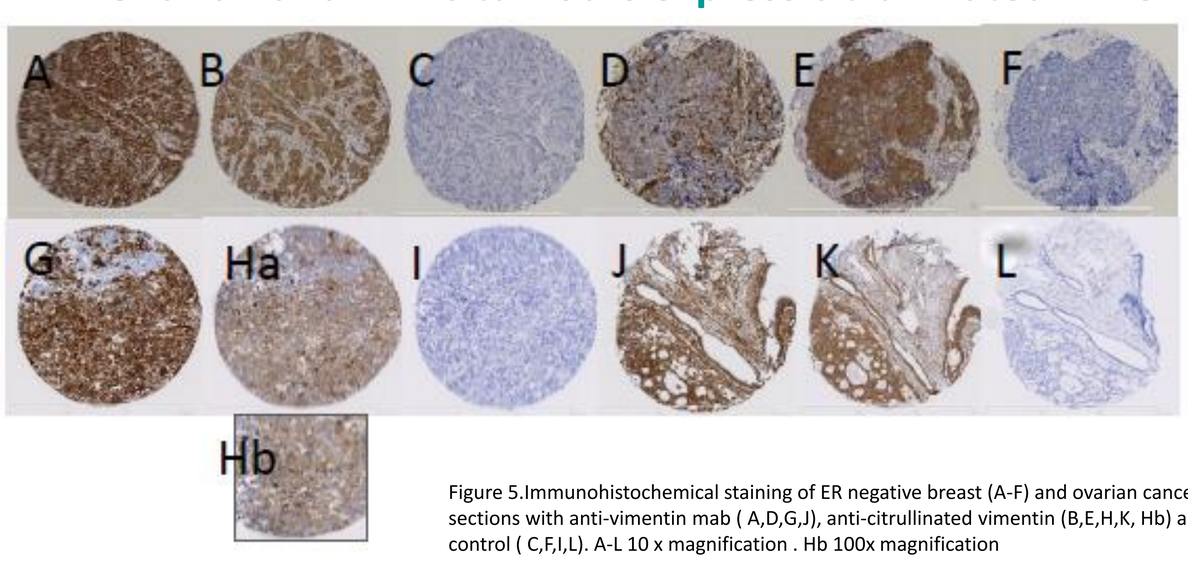


Figure 5.Immunohistochemical staining of ER negative breast (A-F) and ovarian cancer (G – L) sections with anti-vimentin mab (A,D,G,J), anti-citrullinated vimentin (B,E,H,K, Hb) and isotype control (C,F,I,L). A-L 10 x magnification . Hb 100x magnification

CONCLUSIONS

- **Healthy donors show Th1 responses to Modi-1 peptides and share a common HLA allele, HLA-DP4**
- **Th1 responses to Modi-1 peptides can be stimulated in HLA-DP4 and HLA-DR4 transgenic mice in combination with TLR agonists**
- **IL-10 responses to Modi-1 peptides are rapidly stimulated in the absence of costimulation (IFA) suggesting a pre-exisiting response but this does not protect against tumour challenge**
- **Combination of Modi-1 peptide with TLR agonist polarises a Th1 response and provide efficient tumour therapy in melanoma, pancreatic, lung and ovarian cancer models**
- **Citrullinated vimentin is expressed by ovarian and TNBC tumours suggesting them as suitable targets for the Modi-1 vaccine**

References:

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